1

NOVEL ANTI-VIRAL COMPOUNDS

FIELD AND BACKGROUND

The present invention concerns compounds with anti-viral activity, including
5 novel inhibitors of the 3C, 2A and 3C-like cysteine proteases or picornaviridae and
related viruses (see Gorbalenya, Prospectives Drug Discovery Design, 6: 64-86, 1996).

The picornavirus family encompasses single-stranded positive-sense RNA
viruses, encapsulated in a protein capsid (Reviewed in Rucekert, 1996, in Fields,
Virology, 609-654). These viruses cause a wide range of diseases in humans and other
10 mammals, including the common cold (rhinoviruses), gastro-intestinal ailments
(enteroviruses, such as coxsackievirus and echovirus), poliomyelitis (the enterovirus
poliovirus), heart diseases (cardioviruses), hepatitis (hepatitis A) and foot and mouth
disease (aphtoviruses). Rhinoviral common cold is of specifically noted medical and
economical significance, due both to its ubiquity (the major cause of acute illness in the
15 united states) and to thee debilitating effect of the disease and consequential loss of
work days (see McKinlay, *Ann. Rev. Microbiol.*, **46**:635-54, 1992). Related viral
families of the picornavirus supergroup, such as flaviviridae and potyviridae, cause
various diseases in agriculturally significant crops, such as potatoes (see Ryan, J. Gen.
Virol., 4:699-723, 1997).

20 After infection of the host cell, the picornaviral RNA is translated into a single
polyprotein which has auto-catalytic proteolytic activity, cleaving itself into mature
viral proteins. The 2A and 3C proteolytic enzymes, which are part of the picornaviral
autocatalytic polyproteins, are responsible for these cleavages. The 2A protease cleaves
co-translationally between the structural and non-structural protein precursors and the
25 3C protease cleaves most of the remaining sites post-translationally.

The 3C and in some cases 2A, proteases are responsible for maturation of the
picornavirus and are crucial for completion of the picornaviral life cycle. As a result,
the 3C protease has been a prime target for extensive structural and mechanistic
investigations during the last few years, and its mechanism of action and structural

2

features has been determined (Kreisberg *et al*, *Organic Reactivity: Physical and Biological Aspects*, 110-122 (1995)).

The 3C and 2A proteases have somewhat different specificities for substrates. Furthermore, the two proteases have different mechanisms of action because the 2A
5   protease requires zinc, while the 3C protease does not.

Attempts to find treatments for diseases caused by a picornavirus species have been largely directed towards finding inhibitors for the 3C proteases, since such inhibition would prevent production of new virions as there are no native cellular proteases which can replace the cleavage activity of these viral proteases. Therefore,
10 finding an efficient inhibitor of 3C picornavirus protease activity, or of other viral activity, would be valuable for the treatment of a large number of viral diseases, both in humans and in lower animals.

The first inhibitor of the 3C protease to be found by screening was thysanone, an antibiotic compound obtained from *Thysanphora penicilidies* (Singh *et al*, *Tetrahedron*
15 *Lett.*, **32**: 5279-82 (1991)). However, this compound was not developed into a pharmaceutical composition, since it exhibited modest protease inhibitory activity and was found to be an inhibitor of the elastase enzyme present in erythrocytes.

Two additional antibiotic compounds, of fungal origin, termed citrinin hydrate and radicinin were obtained by screening over 20,000 microbial extracts (Kadam *et al*,
20 *J. Antibiotics* 7: 836-839 (1994)). These two compounds were weaker inhibitors of picornaviral proteases than thysanon. In this same screening process, a new compound termed kalafungin, which is also an antibiotic compound, was discovered by structural comparison to radicinin. Kalafungin was found to be a better inhibitor, by three orders of magnitude, than radicinin and citrinin hydrate (McCall *et al*, *Biotechology*, **12**: 1012-
25 1016 (1994)). However, none of these inhibitors has yielded a clinically useful compound, combining high anti-viral activity but low toxicity.

Another group of inhibitors, substituted isatins, has also been examined (S.E. Webber *et al.*, *Med. Chem.*, **39**:5072-5082, 1996). Certain members of this group show significant inhibition of 3C proteases with concentrations in the nmolar range, but are

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highly toxic. Other members of the group are relatively non-toxic, but have poor anti-viral activity. Thus, none of the background art compounds combine strong protease inhibitor or anti-viral effects with low toxicity.

There is therefore an unmet medical need for new compounds which have strong
5 anti-viral activity against the picornaviruses.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide novel derivatives of the known differentiation factor DIF1 which have anti-viral and protease inhibitory activity. It is
10 another object of the present invention to provide pharmaceutical compositions which use these derivatives, as well as DIF1, to treat a disease caused by a picornavirus. It is yet another object of the present invention to provide methods of use of these pharmaceutical compositions to treat a disease caused by a picornavirus. It is still another object of the present invention to provide a method for the detection of a
15 picornavirus.

According to the present invention, there is provided a compound of the formula (I):
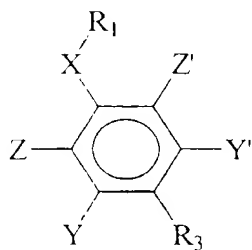
(I)

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wherein:

X is selected from the group consisting of C=O, S=O, C=S, (C=O)-N, (C=O)-O and
(C=O)-S;

25 R_1 is selected from the group consisting of:

4

(i) hydrogen, hydroxyl or a hydrocarbon chain of from about 1 to about 10 carbons long selected from the group consisting of saturated, unsaturated and fluorinated, wherein said hydrocarbon chain is unsubstituted or substituted with at least one R^{11}, wherein R^{11} is selected from the group consisting of:

(ia) C_1-C_4 alkyl, C_2-C_4 alkenyl, C_3-C_8 cycloalkyl, C_6-C_{10} bicycloalkyl, benzo-fused or aryl which may be substituted or unsubstituted;

(ib) halogen, cyano, nitro, amino, hydroxy, adamantyl, carbamyl, carbamyloxy or keto;

(ic) an oligopeptide of 1-3 amino acid residues; and

(id) $NR^{13}R^{14}$, CO_2R^{13}, $O(C=OR^{13})$, SO_2R^{14}, SOR^{14}, $(C=O)NR^{13}R^{14}$, or $NR^{14}(C=O)R^{13}$;

wherein:

R^{13} is selected from the group consisting of hydrogen, phenyl, benzyl, C_1-C_6 alkyl and C_3-C_6 alkoxyalkyl; and

R^{14} is selected from the group consisting of hydrogen, hydroxyl, C_1-C_4 alkyl and benzyl;

(ii) an oligopeptide of 1 to 5 amino acids or a peptidomimetic molecule having substantially similar binding properties as the oligopeptide; and

(iii) C_3-C_8 cycloalkyl and C_6-C_{10} bicycloalkyl, C_3-C_7 cycloalkylmethyl, a benzo-fused phenyl, or a C_5-C_8 heterocyclic ring system including at least one nitrogen, oxygen or sulfur atom, which may be additionally substituted with R^{11} as defined above;

R_3 is selected from the group consisting of:

(i) hydrogen, phenyl, hydroxyl, C_1-C_{12} hydrocarbon chain or O-C_1-C_{12} hydrocarbon chain which may be additionally substituted with at least one R^{11} as defined above; and

(ii) an oligopeptide of 1 to 3 amino acids, an oligopeptide of 1 to 3 amino acids joined to the backbone by an oxygen, or a peptidomimetic;

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Z is selected from the group consisting of hydroxyl, sulfhydryl, amino, carboxyl and NHR^{11}, wherein R^{11} is defined as above;

Z' is selected from the group consisting of:

(i)   hydroxyl, amino, carbamido, carbamyl, carbamyloxy or halogen;

(ii) hydrogen; and

(iii) C_1-C_4 alkyl, C_2-C_5 alkenyl, C_3.C_8 cycloalkenyl, or C_1-C_5 alkoxy which may be additionally substituted with at least one R^{11} as defined above;

Y and Y' are independently selected from the group consisting of:

(i)   hydrogen, halogen, C_1-C_4 haloalkyl, or C_1-C_4 haloalkoxy;

(ii) carbamyl, carbamido, cyano, keto, vinyl, sulfoxide, nitro, C_1-C_3 alkylsulfonyl, sulfonate or sulfone; and

(iii) C_1-C_3 alkyl which may be additionally substituted with at least one R^{11} as defined above; and

(iv) an oligopeptide of 1 to 3 amino acids or a peptidomimetic; and

alternatively Z' and R_1 collectively form a ring system selected from the group consisting of:

(a) C_5-C_8 carbocyclic ring which may be saturated or unsaturated, and which may be additionally substituted with at least one R^{11} as defined above; and

(b) C_5-C_{10} heterocyclic ring system which may be saturated or unsaturated and which includes at least one nitrogen, oxygen or sulfur atom, and which may be additionally substituted with at least one R^{11} as defined above;

and pharmaceutically acceptable salts thereof;

with the proviso that when X-R_1 is a fluorinated keto acyl, Z is hydrogen.
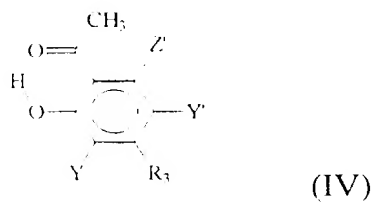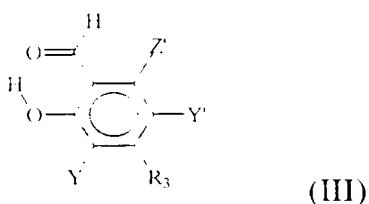
According to preferred embodiments of the present invention, R_1 and R_3 are each independently oligopeptides, peptidomimetic molecules of these oliopeptides, or substituted or unsubstituted, saturated or unsaturated, hydrocarbon chains which may be straight, branched or cyclic.

6

Examples of specific compounds according to the present invention are compounds of the following formulae II - XIX, it being noted that these compounds are all specific examples of compounds of formula I :
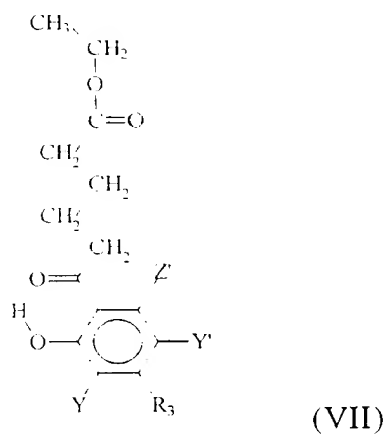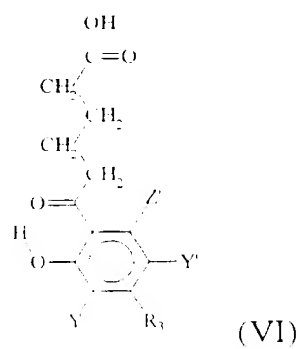

(II - DIF1)
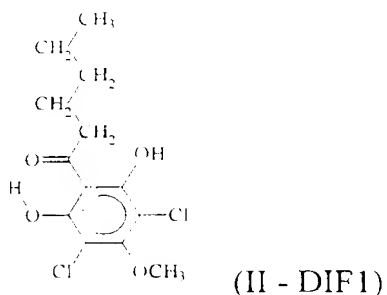
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It should be noted that DIF1 is a known molecule, and that furthermore in this rendition, $X-R_1$ is $(C=O)-(CH_2)_4-CH_3$, Z and Z' are both hydroxyl, Y and Y' are both Cl and R_3 is $O-CH_3$. However, although the molecule itself is known, the novel effects and uses of DIF1, as a viral protease inhibitor and as an anti-viral compound, which are

10 disclosed herein were neither suggested nor taught in the prior art. Indeed, in the prior art DIF1 is known only as a differentiation factor.

(III)

15   (IV)

7

(V)

(VI)

(VII)

OH

O=
H
O— —Y'

Y   R₁   (VIII)

CH₃
CH₂
C=O
CH₂
CH₂
O=
H
O— —Y'

Y   R₁   (IX)

OH—
O=   Z'
H
O— —Y'

Y   R₁   (X)

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OH—
O=   Z
H
O— —Y'

Y   R₁   (XI)

O=   O
H
O— —Y'

Y   R₁   (XII)

(XIII)
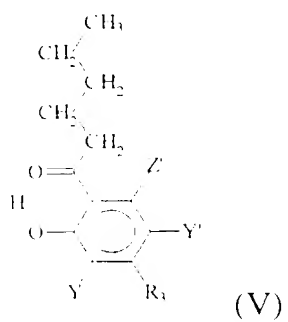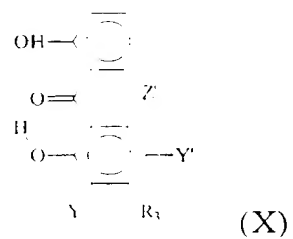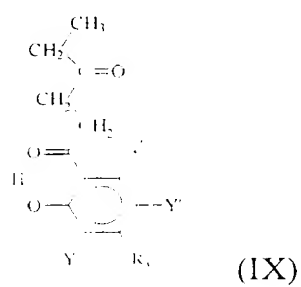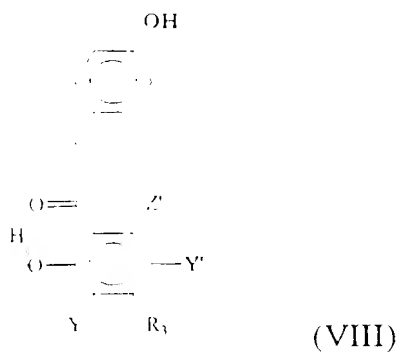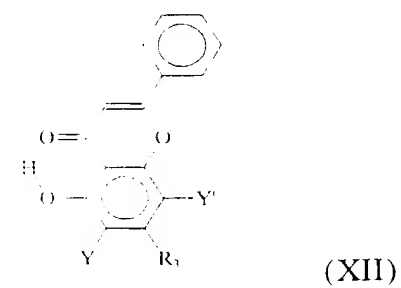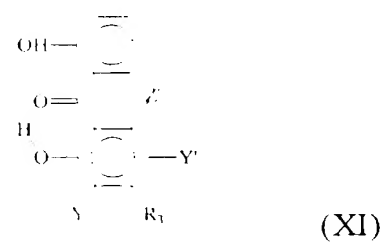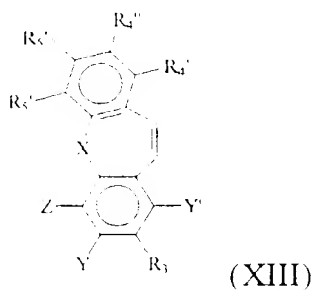
It should be noted that R_4', R_4'', R_5' and R_5'' are all preferably R^{11} as defined herein.

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(XIV)

(XV)

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(XVI)
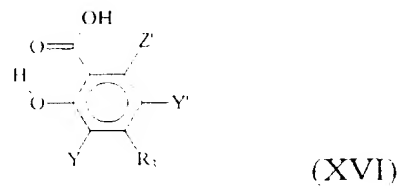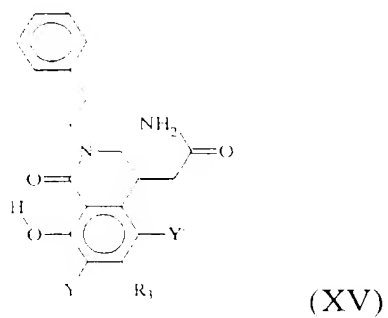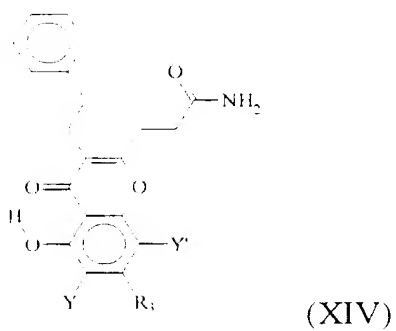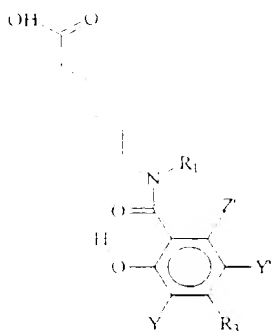
(XVII)

In another embodiment of the present invention, there is provided a composition for treating a disease caused by a picornavirus species comprising a pharmaceutically

5   effective amount of a compound of formula I in combination with a pharmaceutically acceptable carrier.

In yet another embodiment of the present embodiment, there is provided a method of manufacturing a medicament for treating a disease caused by a picornavirus species, comprising the step of placing a pharmaceutically effective amount of a compound of

10   formula I in a pharmaceutically acceptable carrier.

According to another embodiment of the present invention, there is provided a method for the treatment of a disease caused by a picornavirus species in a subject, including the step of administering a pharmaceutically effective amount of a compound of formula I to the subject.

15   In yet another embodiment of the present invention, there is provided a composition for inhibiting a 3C protease or a cysteine protease with an active site similar to the active site of a 3C protease, the composition including a compound of formula (I). Hereinafter, the term "cysteine protease with an active site similar to the active site of a 3C protease" or "3C-like protease" includes cysteine proteases with an

20   active site having a catalytic dyad of Histidine and Cysteine.

According to still another embodiment of the present invention, there is provided a method for determining the presence of a picornavirus species in a sample including the steps of: (a) conjugating the compound of formula I to a detectable label to form a labelled compound; (b) contacting the labeled compound with the sample under

conditions enabling binding between the inhibitor and viral proteins; and (c) determining whether any proteins in the sample are bound to the inhibitor, a positive answer indicating the presence of a picornavirus species in the sample.

DESCRIPTION OF THE INVENTION

Unexpectedly, it has been found that the compound identified as DIF1 is a specific inhibitor of the picornavirus 3C protease, and does not inhibit other proteases normally present in the cell. Even more unexpectedly, it has been found that various derivatives and isomers of DIF1 have stronger inhibitory activity than the native compound. Furthermore, these derivatives and isomers also display strong anti-viral activity in whole-cell assays and show substantially low toxicity.

Without desiring to be limited to a single mechanism, the compounds of the present invention appear to have at least part of their anti-viral effect by selectively binding to the picornaviral proteases, essentially in a similar manner as the virally coded natural substrate of the proteases. However, since these compounds are not cleaved, they remain at the binding site of the enzyme, preventing it from cleaving the natural substrate. This competition serves to inhibit viral maturation and thus to inhibit disease progression *in vivo*.

The parent compound of the inhibitors of the present invention, DIF1 (Differentiation Inducing Factor 1), is chemically defined as 1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-hexan-1-one (Morris *et al*, *Nature*, **328**: 811-814 (1987)). Iso-DIF1, a related compound, is chemically defined as 1-(3,5-dichloro-2,4-dihydroxy-6-methoxyphenyl)-hexan-1-one. DIF1 has been isolated from the cells of the slime mold *Dictyostelium discoideum*. This mold grows in the single-cell form, but when starved the cells aggregate to form a multi-cellular organism which transforms itself into a migrating 'slug'. Within this slug there is a simple spacial pattern of cell differentiation with prestalk cells in the anterior and prespore cells in the posterior. This pattern reflects the final stalk/spore propagation of the mature fruiting body. Stalk-cell differentiation *in vitro* can be induced by DIF1 (Town *et al*, *Nature*, **202**: 717-719

(1976); Brookman *et al, Dev. Biol.,* **91**: 191-196 (1982)). Recently, it has been found that DIF1 induces differentiation of murine and human undifferentiated leukaemia into haemoglobin-synthesizing erythrocyte-like cells (Asahi *et al, Bioch. Biophy. Res. Com.,* **208** (3): 1036-1039 (1995)). The prior art recognizes the role of DIF1 as a general
5 differentiation factor in a number of species.

The particularly strong activity of DIF1 as a picornavirus protease inhibitor is very unexpected, however. Furthermore, inhibitors of 3C picornavirus protease have also been shown to be efficient inhibitors of 2A picornavirus protease, so that the inhibitors of the present invention may also be used to inhibit the 2A protease (Yiu S.F.
10 *et al. Virology,* **J**, 615-625 (1991)).

As will be no doubt appreciated by the person versed in the art, Formula 1 above includes a large number of possible compounds, some of which are more effective inhibitors of 3C and 2A proteases than others. Furthermore, other additions can be made to the substituent groups of the compounds of Formula 1 for other types of
15 functionality.

For example, optionally the hydrocarbon of R_3 bears a label such as a radioisotope, a fluorescent label, or a member of the pair biotin/avidin and the like. These detectable labels will enable specific determination of the presence of 3C or 2A picornavirus proteases in a sample indicative of a picornaviral infection. R_3 may also
20 bear a cytotoxic moiety capable of destroying the cell harboring the picornavirus, or a specific marker which promotes intracellular degradation. In such a case the specific compound of the invention is used to target the cytotoxic moiety, or degradation marker, specifically to the picornavirus.

Preferred compounds of the present invention include the following compounds
25 of the formula (I):

$$\begin{array}{c} R_1 \\ X \quad Z' \\ Z \!-\!\bigcirc\!-\! Y' \\ Y \quad R_3 \end{array}$$
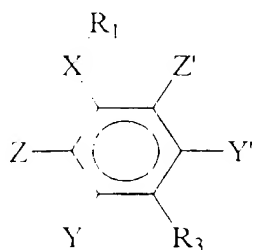
(I)

wherein X is preferably selected from the group consisting of $C=O$ and $(C=O)-N$.

5   Preferably, Z is hydroxyl.   Also preferably, Z' is selected from the group consisting of:

(i)   hydroxyl, amino, carbamido, carbamyl, carbamyloxy or halogen;

(ii)   hydrogen; and

(iii) C_1-C_4 alkyl, or C_1-C_3 alkoxy which may be additionally substituted with at
10   least one R^{11} as defined above.   More preferably, there are 0-2 R^{11} groups as defined above.

Alternatively, R_3 can be an oligopeptide of 3 to 12 amino acid units, preferably an oligopeptide capable of binding to the 3C active site. Various examples of R_3 suitable as substitutes in compounds against specific viruses are depicted in Figure 2 for
15   purposes of illustration only.   Optionally, R_3 can be a fragment of the amino acid sequences depicted in Figure 2. R_3 can also be a peptidomimetic molecule.

In accordance with one embodiment of the invention R_1 is selected from the group consisting of hydrogen, hydroxyl or a hydrocarbon chain of from about 1 to about 10 carbons long selected from the group consisting of saturated, unsaturated and
20   fluorinated, wherein the hydrocarbon chain is unsubstituted or substituted with at least one R^{11}, wherein R^{11} is preferably selected from the group consisting of:

(ia) C_1-C_4 alkyl, or aryl which may be substituted or unsubstituted;

(ib)   halogen, cyano, nitro, amino, hydroxy, adamantyl, carbamyl, carbamyloxy or keto;

25   (ic)   an oligopeptide of 1-3 amino acid residues; and

14

(id) CO_2R^{13}, $O(C=OR^{13})$, $(C=O)NR^{13}R^{14}$, or $NR^{14}(C=O)R^{13}$;

wherein:

R^{13} is selected from the group consisting of hydrogen, phenyl, benzyl, C_1-C_6 alkyl and C_3-C_6 alkoxyalkyl; and

5   R^{14} is selected from the group consisting of hydrogen, hydroxyl, C_1-C_4 alkyl and benzyl.

Preferably, there are from 1 to 3 R^{11} groups.

According to another embodiment of the present invention, R_1 is selected from the group consisting of C_3-C_8 cycloalkyl and bicycloalkyl, C_3-C_7 cycloalkylmethyl, C_7-

10    $_{10}$ arylalkyl, which may be additionally substituted with R^{11} as defined above.

Alternatively, R_1 is an oligopeptide of 1 to 5 amino acid units capable of specifically recognizing and binding to the active site of the species-specific 3C protease against which the inhibitor is directed, since the active site of each 3C protease

15   differs slightly form one type of picornavirus to the other. Generally speaking, the oligopeptide should be able to mimic the amino acid sequence of the viral proteins to which the 3C protease binds; some of these amino acids are conserved and common to all picornaviruses, while others vary between the sub-families, and species. This description is true for all oligopeptides disclosed as substituents herein.

20   Examples of R_1 sequences suitable in accordance with the second embodiment of the invention are specified in Figure 1, wherein each sequence is depicted next to the name of the virus which 3C protease the respective R_1-carrying compound may inhibit.

R_1 can optionally be a fragment of at least 3 amino acids of the sequences specified in Figure 1. Hereinafter, the term "amino acid" is understood to include the

25   20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including for example hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine.

15

Furthermore, the term "amino acid" includes both D- and L-amino acids. The term "oligopeptide" refers to a series of amino acids linked by peptide bonds.

Other examples of suitable amino sequences of various picornaviruses may be constructed according to the teachings of Cordingley *et al J. Biol Chem.*, **265** (16):
5   9062-9065 (1990).

Suitable oligopeptides of 1 to 5 amino acids may be found by immobilizing an appropriate 3C picornavirus protease by attaching it to beads, for example, and then determining which peptides were able to specifically bind to the immobilized proteases. Such oligopeptides are suitable for serving as R_1 in the inhibitor of the second
10   embodiment of the invention.

According to other embodiments, R_1 may be a peptidomimetic molecule, for example selected from one of the molecules stipulated in Figure 2 prepared by peptidomimetic methods, which has similar binding properties to the active site as the oligopeptide of the second embodiment of the invention. Methods for preparing
15   peptidomimetic compounds are well known in the art and are specified in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992).

Specifically, peptidomimetic compounds can be prepared by altering the base structure of a portion of the ideal substrate for the HRV-14 (Human Rhinovirus 14) 3C protease, which has the amino acid sequence EALFQGPLQ. This structure is altered
20   by replacing at least the peptide bond between Q and G with various non-peptidic bonds, such as CH_2-NH, CH_2-S, CH_2-S=O, O=C-NH, CH_2-O, CH_2-CH_2, S=C-NH, CH=CH or CF=CH. The resulting moiety has a sufficiently similar structure to the preferred substrate for good binding to the active site of the protease, but is not readily cleavable by the enzyme, thereby remaining bound and inhibiting its activity.
25   Hereinafter, the term "peptidomimetic" refers to an molecule which is not an oligopeptide, yet has a binding affinity to the protease active site which is at least as good as the native oligopeptide.

Alternatively and preferably, Z' and R_1 collectively form a ring system selected from the group consisting of C_5-C_{10} heterocyclic ring system which may be saturated or

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16

unsaturated and which includes at least one nitrogen, oxygen or sulfur atom, and which may be additionally substituted with at least one, and more preferably 1-3, R^{11} as defined above.

The compounds of the present invention have the advantage of strong selective
5 protease inhibitory activity without substantial toxicity. Furthermore, the compounds of the present invention also display strong anti-viral activity. However, the mechanism of action of these compounds could also involve inhibition of other viral processes and stages of the virus life cycle. Hereinafter, the term "anti-viral" includes any effect which substantially interrupts, prevents or reduces any viral activity and/or
10 stage of the virus life cycle, or which reduces or prevents infection by the virus in a subject, such as a human or lower animal, without regard to a particular mechanism of action such as inhibition of 3C protease activity.

Nowhere in the prior art is it taught or suggested that the compounds of the present invention have significant anti-viral activity, or that these compounds are 2A or
15 3C protease inhibitors. Indeed, the compounds of the present invention can be readily distinguished from those of the prior art both by their basic structure and by their efficacy. For example, as noted above, these compounds display greater anti-viral activity than DIF1. In U.S. Patent No. 5,514,778, IC_{50} anti-viral inhibitory activity requires mM quantities. By contrast, many of the compounds of the present invention
20 have anti-viral activity with an IC_{50} of only μM quantities, 3 orders of magnitude more potent. Not surprisingly, the structures of the two groups of compounds are also completely different.

Thus, the compounds of the present invention show clear advantages over prior art compounds.

25

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows amino acid sequences of oligopeptides capable of being formed into R_1 in accordance with an embodiment of the invention. Left column - the name of the

virus as depicted by the GCG-code in the database. Right column - the appropriate

amino acid sequences in one letter code;

~~FIG. 2A gives examples of peptidomimetic moeities for R$_1$; and~~

FIG. 2B gives examples of compounds of the present invention with a

~~peptidomimetic moeity.~~

DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to compounds which have strong anti-viral activity

against picornavirus species. Furthermore, pharmaceutical compositions and methods

of treatment of diseases caused by a picornavirus species are also disclosed.

Synthesis of Compounds of the Present Invention

The compounds of the present invention can be synthesized according to

procedures which are well known in the art.

A general example of a method of synthesis of the compounds of the present

invention is as follows. Compounds of formula (I), where X is C=O, Z is hydroxyl, R$_3$

is OH and Z', Y and Y' are hydrogen, can be prepared with any R$_1$ group by

implementation of the Hoesch reaction between resorcinol and the appropriate

commercially-available nitrile, R$_1$-CN (Ruske, Friedel-Crafts and Related Reactions, ed

by G.A. Olah, 1965, pp. 383-497). Likewise the Hoesch reaction performed with

fluoroglucinol with the appropriate nitrile yields compounds of formula (I) where X is

C=O, Z , Z' and R$_3$ are hydroxyl, and Y and Y' are hydrogen. Similarly, the same

reactions performed with the appropriate nitrile and meta (lower) alkoxy-phenols, such

as 3-hydroxyanisole or 5-methoxyresorcinol, also yields compounds of formula (I)

where X is C=O, Z is hydroxyl, Z' is lower alkoxy, R3 is hydrogen or hydroxyl and Y

and Y' are hydrogen.

Compounds of formula (I), where X is C=O, Z is hydroxyl and one or both of Y

and Y' are Cl can be readily prepared by chlorinization of corresponding compounds,

where either or both of Y and Y' are hydrogen, by using the reagent sulfuryl chloride (Masilamani and Rogic, *J. Org. Chem.*, **46**:4486-4489, 1981).

The following specific examples of particular methods of synthesis are intended for illustrative purposes only and are not meant to be limiting.

5

Compound 109: Formula IV, Z'=OH, Y=Y'=Cl, R$_3$=H

A solution of 2,6-dihydroxyacetophenone (152mg, 1 mmol) in absolute methanol (1 ml), was diluted in anhydrous dichloromethane (10 ml). To the resultant clear solution was added dropwise a 1M solution of sulfuryl chloride in anhydrous dichloromethane (2 ml) at room temperature. After stirring at room temperature for 1.5 hours, the solvents were evaporated and the residue was purified by silica gel column chromatography with hexane : dichloromethane (30:70), to provide 80 mg of a brown-orange solid, M.P. 147.5°C. ^{1}H NMR (DMSO-^{6}d, 200 MHz) : δ 7.67 (s, 1H), 2.63 (s, 3H), MS (EI$^+$): 205.0 (M$^+$-CH$_3$, 100%), 220.0 (M$^+$, 47%).

Compound 21: Formula V, a 4:1 Mixture
of Compounds 108 and 107, Respectively

Dihydroxyanisole (1.113g ,7.9 mmole), hexanonitrile (1.60 ml, 13.2 mmol) and zinc chloride (700 mg) were dissolved in 50 ml of sodium-dried ether. The stirred solution was saturated with a steady stream of dry hydrogen chloride gas and after 5 minutes turned milky. 10 minutes later, a viscous orange oil separated, and the mixture was left overnight. The colorless solution was decanted and the oil was taken in 50 ml of ice-cold water. The resultant clear orange-red solution was extracted with ether (2 × 50 ml) and the aqueous phase was boiled on a hot-plate and concentrated to half the original volume (ca. 30 ml). At this point, the solution became turbid, and upon cooling a brown-orange solid separated (810 mg), M.P. (air-dried) 100°C. Two products were apparent by TLC [(chloroform:ether 1:1), R$_f$ = 0.68 (major), R$_f$=0.76

(minor) : (dichloromethane), R_f = 0.10 (major), R_f=0.33 (minor)]. ^{1}H NMR (CD$_3$OD, 200 MHz) : δ 5.95 (d, 2H: *major*), 5.90 (s, 2H: *minor*), 5.87 (d, 1H: *major*), 3.85 (s, 3H: *major*), 3.76 (s, 3H: *minor*), 2.99 (t, 2H: *minor*), 2.94 (t, 2H: *major*), 1.63 (m, 2H), 1.34 (m, 4H), 0.92 (3H, t). The product ratio was 4.3:1, as determined by NMR integration at δ 3.85 (*major*) vs δ 3.76 (*minor*), MS (EI$^+$): 238.1 (M$^+$, 18%), 167.0 (M$^+$-C$_5$H$_{11}$, 100%).

Compounds 107 and 108: Formula V

Z'=OH, R$_3$=OCH$_3$, Y=Y'=H (107)

Z'=OCH$_3$, R$_3$=OH, Y=Y'=H (108)
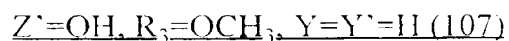
750 mg (3.15 mmol) of the isomeric methoxy-hexanoresorcinones mixture (compound 21) was loaded on a silica gel column equilibriated with dichloromethane. Fractions 6-7 (eluted with dichloromethane) contained pure (TLC) of the minor isomer. Evaporation of the solvent yielded 78 mg of white solid, corresponding to compound 107, M.P. 121°C. TLC (dichloromethane), R_f=0.33. ^{1}H NMR (CD$_3$OD, 200 MHz) : δ 5.90 (s, 2H), 3.76 (s, 3H), 3.03 (t, 2H), 1.65 (t, 2H), 1.35 (m, 4H), 0.91 (t, 3H). MS (EI$^+$): 167.1 (M$^+$-C$_5$H$_{11}$, 100%), 238.2 (M$^+$, 15%). Fractions 10-12 (eluted with 4:1 ethyl acetate:dichloromethane) contained pure (TLC) major isomer. Removal of the solvents yielded 384 mg of grayish solid, corresponding to compound 108, M.P. 109°C. TLC (dichloromethane), R_f=0.10. ^{1}H NMR (CD$_3$OD, 200 MHz) : δ 5.94 (d, 1H), 5.87 (d,1H), 3.84 (s, 3H), 2.93 (t, 2H), 1.62 (t, 2H), 1.34 (m, 4H), 0.92 (m , 3H). MS (EI$^+$): 238.2 (M$^+$, 39%), 167.1 (M$^+$-C$_5$H$_{11}$, 100%).

Compound 15:

Formula V, Z'=OH, R$_3$=OCH$_3$, Y=Y'=Cl

A stirred solution of 2,6-dihydroxy-4-methoxyhexanophenone (cf. compound 107 : 28 mg, 0.12 mmol) in absolute methanol (0.25 ml), was diluted in anhydrous

dichloromethane (10 ml) at room temperature. A 1M solution of sulfuryl chloride in anhydrous dichloromethane was added dropwise (0.26 ml, 2.2 mmol), and the color of the resultant mixture was observed to change from faint yellow to bright vivid yellow within seconds. 20 minutes later, the solvents were evaporated and residual sulfuryl chloride was removed in vacuo, resulting in yellow crystals (38 mg), M.P. 101°C. ^{1}H NMR (CDCl$_3$) : δ 10.32 (s, 2H), 3.98 (s, 3H), 3.13 (t, 2H), 1.71 (t, 2H), 1.35 (m, 4H), 0.91 (t, 3H) [identical to spectrum described by Masento *et al.*, 1988, *Biochem. J.* 256: 23-28]. MS (EI$^+$): 306.0 (M$^+$, 21%), 235.0 (M$^+$-C$_5$H$_{11}$, 100%).

Compound 16:

Formula V, Z'=OCH$_3$, R$_3$=OH, Y=Y'=Cl

2,4-dihydroxy-6-methoxyhexanophenone (cf. compound 108: 10 mg, 0.04 mmol) was reacted with sulfuryl chloride in a manner analogous to that described in compound 15 above. The product was vaccuum-dried, resulting in orange crystals (12 mg). ^{1}H NMR (CDCl$_3$) : δ 14.02 (s, 1H), 6.51 (s, 2H) (s, 1H), 3.91 (s, 3H), 3.05 (t, 2H), 1.69 (t, 2H), 1.32 (m, 4H), 0.89 (t, 3H).

Compound 22:

Formula V, Z'=OH, R$_3$=OH, Y=Y'=H

The Hoesch reaction was employed following the general procedure of Howells and Little (Howells, H.P. and Little, J.G., 1932, *J. Am. Chem. Soc.* 54: 2451-2453), with the addition of an initial reaction of hexanitrile in the absence of aromatic reactant, a means of improving the yield (see March, Advanced Organic Chemistry, 1968, p. 424). Through a stirred solution of hexanonitrile (1.37 ml, 11.0 mmol) and zinc chloride (600 mg) in 30 ml of dry ether containing 1M hydrogen chloride, was passed a stream of dry hydrogen chloride gas at room temperature. After 0.5 hour, the following were added: phloroglucinol (1.0g ,7.9 mmole), sodium-dried ether (18 ml) and zinc

chloride (400 mg), and the mixture was saturated with dry gaseous hydrogen chloride. During the next 0.5 hour, the solution turned milky, and red oily droplets appeared. In the course of another 0.5 hour, the mixture cleared. The resultant red-wine solution was left overnight, and subsequently diluted by the addition of ice-cold water (40 ml). The

5   aqueous phase was extracted with ether (3 × 30 ml), and boiled on a hot-plate. After a few minutes, a gray sediment appeared. The solution was further boiled, the initial volume being reduced by half. Upon cooling, 1.13 g of a brown solid was obtained. ^{1}H NMR (CD$_3$OD, 200 MHz) : δ 5.80 (s, 2H), 3.02 (t, 2H), 1.65 (m, 2H), 1.34 (m, 4H), 0.92 (3H, t).

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22

Compound 23:

Formula IV, Z'=OCH$_3$, R$_3$=OH, Y=Y'=H

Dihydroxyanisole (0.500 g, 3.57 mmol), zinc chloride (170 mg) and acetonitrile (0.214 ml, 4.10 mmol) in 35 ml of dry ether were stirred at room temperature. A steady
5 stream of dry gaseous hydrogen chloride was passed through the mixture, and after 10 minutes the solution turned cloudy and a brown oil separated. In the course of another 5 minutes, the solution above the oil cleared.. After 2.5 hours, 25 ml of cold water were added. and the mixture was left overnight. The aqueous phase was extracted with water and concentrated by bioling on a hot-plate. Pink crystals began to form after the volume
10 of the aqueous phase was reduced by half, and accumulated on cooling, resulting in 94 mg, 68 mg of which were recrystallized in water to yield 34 mg of pink crystals, M.P. 201-202°C. TLC (dichloromethane:ether 7:3),. R$_f$ = 0.57. UV, λ=287.3nm (ϵmM=18.5cm^{-1} M^{-1}). ^{1}H NMR (CDCl$_3$, 200 MHz) : δ 12.0 (s, 1H; chelated) [DMSO-d$_6$: δ 13.82 (s, 1H; chelated)], 7.38 (s, 1H, broad) [DMSO-d$_6$: δ 9.2-11.8 (s, 1H;
15 broad)], 5.96 (s, 1H), 5.65 (s, 1H), 3.87 (s, 3H), 2.61 (s, 3H).

Compound 25:

Formula IV, Z'=OCH$_3$, R$_3$=OH, Y=Cl, Y'=H

20 To a solution of 2,4-dihydroxy-6-methoxy acetophenone (cf. compound 23 ; 30mg, 0.16 mmol) in ether (7 ml) was added sulfuryl chloride (13μL, 0.20 mmol)) in ether (70μL) dropwise, over 5 minutes. 2 hours later, a further portion of sulfuryl chloride (13μL, 0.20 mmol) was added directly and after another hour a sediment appeared, and the mixture was left overnight at -20°C. After evaporation of the ether, a
25 white powder remained, which after recrystallization (ethyl acetate) gave 12 mg of yellow odorless crystals (0.500 g, 3.57 mmol), M.P. 187.5-189°C. ^{1}H NMR (DMSO-d$_6$) : δ 14.6 (s, 1H; chelated), 11.4 (s, 1H), 6.19 (s, 1H), 3.83 (s, 3H), 2.56 (s, 3H [integration confirmed in CD$_3$OD]), MS (EI$^+$): 216.0 (M$^-$, 49%), 167.1 (M$^+$-CH$_3$, 100%).

Compound 112: Formula V, Z'=OH, R₃=OH, Y=Cl, Y'=H

To a stirred solution of 2,4,6-trihydroxy-hexanophenone (compound 22 (60 mg, 0.25 mmol) in absolute methanol (0.5 ml) at room temperature, was added
5 anhydrous dichloromethane (10 ml) and a 1M solution of sulfuryl chloride in dichloromethane (0.50 mmol). After 10 minutes, the solvents were evaporated and 17 mg of the residue were purified by column chromatography (dichloromethane) to provide 5 mg. ^{1}H NMR (CD₃OD) : δ 5.49 (s, 1H), 3.11 (t, 2H), 1.68 (t, 2H), 1.37 (m, 4H), 0.93 (t, 3H).

10

Compound 111: Formula IV, Z'=OH, R₃=OH, Y=Y'=Cl

A stirred solution of 2,4,6-trihydroxy-acetophenone (339 mg, 2.0 mmol) in absolute methanol (05 ml), was diluted in anhydrous dichloromethane (10 ml) at room
15 temperature (19°C). A 1M solution of sulfuryl chloride in anhydrous dichloromethane was added dropwise (2 mmol), and the solution was observed to become turbid with a small amount of solid. After 1 hour, another portion of sulfuryl chloride 1M solution in dichloromethane was added (2 mmol), and after 4 more hours, the solvents were evaporated, resulting in a yellowish solid, 600 mg of which was purified by flash
20 chromatography (dichloromethane to 2:1 dichloromethane: ethyl acetate) to yield 86 mg crystalline yellow-orange solid. ^{1}H NMR (CDCl₃) : δ 9.30 (s, 1H), 2.70 (s, 3H).

Compound 105: Formula VIII, Z'=R₃=OH, Y=Cl, Y'=H

25 To a stirred solution of phloretin (40 mg, 0.146 mmol) in absolute methanol (10 ml), at room temperature, was added 292 μL of a 1M solution of sulfuryl chloride in anhydrous dichloromethane (0.292 mmol), and the yellow solution was observed to become brighter. After 1 hour, the methanol was evaporated and the resultant brownish solid (152 mg) was purified by column chromatography (4:1 chloroform:ethyl acetate),

●   ●

24

to yield 15 mg of brown-purple sheaths. ^{1}H NMR (CD$_3$OD) : δ 7.06 (d. 2H), 6.69 (d. 2H). 5.49 (s. 1H). 3.34 (t. 2H). 2.88 (t. 2H).

Compound 100: Formula XII, R$_3$=OH, Y=Y'=Cl

5   A solution of sulfuryl chloride (0.35ml. 2.15mmol) in dichloromethane (5ml) was added to 250mg Chrysin (5,7-dihydroxyflavone; 0.984mmol), and the resultant slurry was stirred vigorously at room temperature. After 30 minutes, 0.20 ml of a solution of pyridine (1.24 mmole) in chloroform was added dropwise and the mixture was allowed to stir for a further 3 hours. After removal of solvents, diethyl ether was

10   added, resulting in the sedimentation of a flaky yellow solid (210 mg). M.P. 301°C. ^{1}H NMR (DMSO-^{6}d. 200 MHz) : δ 8.21 (dd. 2H). 7.60 (m. 3H). 7.20 (s. 1H). MS (EI$^-$): 322.1 (M$^+$. 100%).

Activity of Compounds of the Present Invention

The following examples of the activity of the compounds of the present invention

15   are meant to be illustrative and are not intended to be limiting in any way.

Example 1

Inhibition of 3C activity by DIF1

The ability of DIF1 to inhibit the cleavage activity of the 3C protease was measured.   DIF1 strongly inhibited the ability of 3C protease to cleave the test

20   substrate. The experimental method was as follows.

First, the synthesis of DIF1 was carried out according to the procedure of Masento et al., Biochem. J., **25b**, 23-28 (1988).

Next, the inhibition of 3C protease activity was measured by using a cleavage assay. A synthetic N-acetylated 10-mer peptide possessing the consensus sequence for

25   cleavage by Rhinovirus protease 3C (Queens's University, Ontario) was used as a substrate. Specific cleavage of this peptide [(N-Ac)Arg-Ala-Glu-Leu-Gln-Gly-Pro-Tyr-Asp-Glu] by HRV 3C provided two pentapeptides. In a typical experiment 80 µg of peptide, dissolved in 100 mM TRIS (pH 8) and 100 mM NaCl, was incubated at 35°C with 2.5 µg of HRV 3C protease with and without DIF1, to give a total volume of 30µl.

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In both cases 3C was suspended in a buffered solution (40mM Tris-HCL, pH 8.0, 100 mM NaCl, 1 mM DTT, 1 mM EDTA). 2.5 mg of DIF1 (dissolved in 1 ml of ethanol) was used, corresponding to a concentration of 270 µM. Inhibitory effects due to the organic solvent were not observed; nevertheless, 10 µl of ethanol were added to the control. Aliquots were drawn at short intervals (within ca. 40 min.) and quenched with an equal volume of 1% TFA in Methanol (HPLC grade).

The digestion mixtures obtained from the cleavage assay were separated by reverse phase HPLC (Pharma Biotech ResourecTM R.P.C. 15µm beads 30 x 6.4 mm) using a 20-80% gradient of 0.1% TFA in MeOH (Carlo-Erba) in 5 minutes (flow rate of 1 ml/min). Simultaneous detection at 280 and 215 nm provided additional resolution (Pharmacia LKB RSD, Uppsala, Sweden), as only one of the pentapeptide cleavage products retains absorption at 280nm, the tyrosine-containing fragment GPYDE.

The degree of 3C protease inhibition by DIF1 was found by integration of peak areas under the 280nm absorption curve. The degree of cleavage was calculated as the ratio of peak areas corresponding to peptide alone and peptide plus GPYDE product. Quantification was based on the initial, linear portion of the absorption curve, which represents less than 20% cleavage.

In the absence of DIF1, there was a marked increase in the level of peptide digestion products, compared to corresponding results obtained in the presence of DIF1. Thus, DIF1 clearly has an inhibitory effect on the cleavage activity of the 3C protease.

Furthermore, the rate of substrate cleavage decreased significantly in the presence of 270 µM DIF1. Ki was calculated to be 220 µM. Thus, clearly DIF1 strongly inhibited the activity of the 3C protease, with an efficacy in the µM range.

An additional assay was performed as follows in order to confirm the above results.

HRV 3C Protease Inhibition Assay - HPLC Method

Materials:

26

Protease: Recombinant HRV-1A 3C protease was produced in E. Coli [BL21(DE3)pLysS] containing the protease gene under the control of an inducible T7 expression vector (Studier, F.W. *et al.*, 1990, in Methods in Enzymology (Goeddel, D.V., ed.), Use of T7 RNA polymerase to direct expression of cloned genes, 185: 60-

5   89). The protease was obtained in soluble form (following cell lysis) by reducing the temperature post-induction (0.4mM IPTG) from 37°C to 25°C, as described by Harris *et al.* (Harris, K.S. *et al.*, 1992, Purification and characterization of poliovirus polypeptide 3CD, a proteinase and a precursor for RNA polymerase, *J. Virol.* 66: 7481-7489). The lysate in 20 mM Tris, pH 8.0, 30 mM NaCl, 1 mM EDTA and 1 mM DTT

10   was kept at -20°C and diluted to a final concentration of 3.3 μM.

Substrate: Peptide of sequence [(N-Ac)Arg-Ala-Glu-Leu-Gln- Gly-Pro-Tyr-Asp-Glu-NH$_2$], representing the consensus sequence for cleavage by HRV-3C, was obtained from Peptide Core Facility (Queen's University, Ontario). Stock solutions of 10 mg/ml were prepared in 100mM Tris-HCl (pH 8.0).

15   Compounds assayed for inhibition were dissolved in 95% EtOH in an amount sufficient to make 8-100 mM stock solutions.

Reactions:

Assayed compound (5.5 μL) and recombinant HRV-3C protease were mixed in buffer containing 50 mM Tris, pH 8.0 and 3 mM dithiothreitol (final EtOH

20   concentration of 5%). A control mixture was prepared in a similar manner, containing 95% EtOH in place of compound. Reactions were initiated, following overnight incubation (30±2°C), by the addition of peptide substrate (final concentration of 810 μM). Aliquots were withdrawn at timed intervals (typically 4, 8 and 12 minutes post-initiation) and quenched by mixing with ice-cold trichloroacetic acid (final

25   concentration of 10%). Quenched aliquots were left on ice for further 30 minutes and centrifuged (14.000 rpm, 5'). The supernatant was further diluted by the addition of 7 volumes of HPLC solvent A (0.1% TFA), prior to loading (80 μL) via autosampler onto HPLC (Waters 626 LC system). Intact peptide [Retention time (R$_t$) 17'] was separated from C-terminal fragment [NH2-Gly-Pro-Tyr-Asp-Glu(NH2), confirmed by

Fab⁻ mass spectrometry ; R_t 8']. on a C18 column (Vydac. 4.6·250 mm, 5 μm particles). The following discontinuous gradient of A to B (20% CH_3CN, 0.1% TFA) was employed at constant flow (1.5 ml/min): i) 0-35% B in 2' at a hyperbolic gradient of steepness 3 (1-11 scale, 6 being linear) ii) A linear gradient of 35-50% B from 2-8',

5   iii) A linear gradient of 50-87.5% B from 8-9' and iv) Isocratic 87.5% B from 9-17'. Tyrosine fluorescence (275 excitation. 310 emission: Bagshaw, 1987) was measured continuously by a Waters 474 fluorescence detector. Integration of peak areas corresponding to control and inhibitor-containing cleavage mixtures (Millenium Chromatorgaphy Manager 2020 software. version 2.10, Waters, Millipore Corp.)

10   provided percent activity values at several time-points. Extrapolation of these values by linear regression provided IC_{50} values directly. Ki values corresponding to inhibitor-enzyme dissociation constants were determined using a Km value of 1.9 mM (Determined by HPLC method).

The HRV-1A 3C protease inhibitory activity of representative compounds of the

15   invention is shown in the table below.

#	Formula	Z	Y	Y^1	R_3	Ki (μM)
1	X (Dioxybenzone)	H	H	H	OMe	4700±900
3	(Juglone)					630±70
15	II(DIF-1)	OH	Cl	Cl	OMe	460±80
16	V (iso-DIF-1)	OMe	Cl	Cl	OH	390±30
17	IX	OH	Cl	Cl	OMe	1670±710
18	VI	OH	Cl	Cl	OMe	860±230
19	VII	OH	Cl	Cl	OMe	690±160
20	V	OH	Cl	Cl	OH	460±60
21	V	OMe/ OH	H	H	OH/ OMe	> 15,000
25	IV	OMe	Cl	H	OH	3000±1500

28

Example 2

Specificity of Inhibition by DIF1

The specificity of DIF1 as an enzyme inhibitor was tested using cleavage assays for a number of other enzymes, including elastase, cathepsin B, chymotripsin, papain

5 and ficin A. Elastase was particularly important because it is present in red blood cells, so that inhibition of its activity could potentially be highly toxic. The results showed that DIF1 does not non-specific inhibition of other the activity of other enzymes. The experimental method was as follows.

The cleavage activity of elastase was determined in 0.1 M Tris-HCl pH 7.0 buffer

10 containing 20 µg/ml enzyme and its synthetic substrate N-succinyl-Ala-Ala-Ala-p-nitroanilide. 50 mM DIF1 did not inhibit the cleavage activity of elastase.

The cleavage activity for cathepsin B was assayed according to the method of Bajowski and Frankfater (*Anal. Biochem.* **68**:119 (1975)). Chymotripsin was assayed in 0.1M KPi buffer pH 7.0 containing 0.04 µg/ml enzyme and 200 µM N-benzoyl-L-

15 tyrosine ethyl ester (from a 20mM stock in dimethyl sulfoxide). The reaction was monitored by spectrophotometry at 256 nm.

Papain and Ficin were incubated for one hour in an activation solution containing 0.1 M KPi buffer pH 7.0, 0.5 mM cysteine, 20mM EDTA and 65 µg enzyme. The assays were carried out by adding 20 µl aliquots from the activation mix to 0.1 M KPi

20 buffer pH 7.0, containing 12.5 nM N-CBZ-Gly-p-Nitrophenyl ester. The reaction was monitored by spectrophotometry at 405 nm. The results showed that all DIF1 concentrations tested were found not to effect the protealytic activity of cathepsin B, chymotripsin, papain and ficin A.

Thus, clearly DIF1 does not show non-specific or undesirable inhibitory activity

25 for other enzymes such as elastase.

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29

Example 3

Derivatives of DIF1

and Inhibition of 3C Protease Activity

The inhibitory activity of a number of derivatives of DIF1 of formula III and IV

5   was determined as described above for DIF1. Ki for the compounds of formula III and

IV was found to be 150 μM and 154 μM respectively, compared to 220 μM for DIF1.

Thus, the derivatives of DIF1 may feature a better enzyme inhibitory activity than the

native compound.

10

Example 4

Inhibition of Virus in

Whole-Cell Assay

The ability of DIF1 derivatives to inhibit viral activity was measured in whole cells,

15   to examine the ability of the compounds to inhibit the viral life cycle. Interestingly,

many of the compounds displayed greater anti-viral activity than inhibition of protease

activity, indicating that the compounds may be acting at a site other than the 3C protease.

The experimental method was as follows.

H1-HeLa cells, obtained from ATCC (F-13824, 1958-CRL) were cultured in MEM

20   (Minimum Essential Medium) supplemented with 10% heat-inactivated fetal calf serum

(FCS) by incubation at 37 C, 5% CO_2 (Biological Industry, Kibbuts Beit HaEmek, Israel).

96 well plates were seeded to 90% confluency. After 12-24 hrs of incubation the cell's

monolayer was wased with phosphate-buffered saline (PBS) and the cells were subsequently

infected with ten-fold dilutions in PBS (log10) of human rhinovirus 14, strain 1059,

25   obtained from ATCC.

For each concentration of compound, three rows of wells containing various dilutions

of virus were used. After 1 h of incubation at room temperatura, medium containing 3%

FCS and various concentrations of compound to be tested was placed in the well.

After 2 days incubation at 34 C, 5% CO_2, the cells were fixed by 10% formaldehyde and

30   stained with methylene blue to determine cell viability. The cytopathic effect of non-viable

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30

cells was arbitrarily set to 100% for the untreated viral infection, so that the effect of each compound on cell viability was determined according to this baseline. Each experiment was performed 5 times for each compound, and the inhibition of cytopathic effect (ICE_{50}) for inhibition of viral activity was calculated. Compounds were tested at concentrations lower

5   than 50% toxic dose (TD_{50}) previously found. ICE_{50} refers to the concentration in which 50% of the cells remain viable after viral infection.

A number of compounds of formula IV were assayed for their anti-viral activity, with the results given in Table 1. Certain substituents were shown to substantially increase the anti-viral activity of the compound, as indicated below.

10

Table 1. Compounds of Formula IV

$\underline{Z'}$	$\underline{Y'}$	$\underline{R_3}$	$\underline{Y}$	$\underline{ICE_{50}\,(\mu M)}$	$\underline{TD_{50}\,(\mu M)}$
H	H	H	H		30
H	H	OH	H		40
H	OH	H	H		5
OH	H	H	H		5
OH	Cl	H	Cl	1250	5000
OH	Cl	OH	Cl		1000
$O-CH_3$	H	OH	H		220
$O-CH_3$	H	OH	Cl	11	22

15   A number of compounds of formula V were also assayed. The results are given below, in Table 2.

31

Table 2. Results for Formula V

Z'	Y'	R$_3$	Y	IC$_{50}$	TD$_{50}$
OH	H	OH	H	155	625
OH	Cl	O-CH$_3$	Cl	7.5 (3)	30
OH	Cl	OH	Cl		1875
OH	H	O-CH$_3$	H		125
O-CH$_3$	H	OH	H	12 (2)	250
O-CH$_3$	Cl	OH	Cl	3 (2)	40
H	H	OH	H	20	170
OH/O-CH$_3$ (4:1 ratio)	H	OH/O-CH$_3$ (4:1 ratio)	H	10	50

Other examples of compounds of other formulas were tested and the results were as follows.

5

Formula	Z'	Y'	R$_3$	Y	ICE$_{50}$	TD$_{50}$
VI	OH	Cl	O-CH$_3$	Cl	20	80
VII	OH	Cl	OH	H		200
VII	OH	Cl	O-CH$_3$	Cl		40
IX	OH	Cl	O-CH$_3$	Cl		80
XII	-	H	H	H		1000
XII	-	Cl	H	Cl	2.5	100
VIII	OH	H	OH	Cl	40	150
VIII	OH	H	OH	H		160
XIV	H	NO$_2$	H	NO$_2$		1875

Thus, clearly many of these compounds of the different formulas have significant anti-viral activity.

Example 5

Compositions of the Present Invention

The compounds of the present invention can be administered to a subject in a number of ways, which are well known in the art. Hereinafter, the term "subject" refers
5 to the human or lower animal to whom the compound was administered. For example, administration may be done topically (including ophtalmically, vaginally, rectally, intranasally), orally, or parenterally, for example by intravenous drip or intraperitoneal, subcutaneous, or intramuscular injection.

Formulations for topical administration may include but are not limited to
10 lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners,
15 diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include but are not limited to sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Pharmaceutically acceptable carriers are well known in the art and are disclosed,
20 for instance, in *Sprowl's American Pharmacy*, Dittert, L. (ed.), J.B. Lippincott Co., Philadelphia, 1974, and *Remington's Pharmaceutical Sciences*, Gennaro, A. (ed.), Mack Publishing Co., Easton, Pennsylvania, 1985.

Pharmaceutical compositions of the compounds of the present invention, or of pharmaceutically acceptable salts thereof, may be formulated as solutions or
25 lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. The liquid formulation is generally a buffered, isotonic, aqueous solution, but a lipophilic carrier, such as propylene glycol optionally with an alcohol, may be more appropriate for compounds of this invention. Examples of suitable diluents are normal

33

isotonic saline solution, standard 5% dextrose in water of buffered sodium or ammonium acetate solution. Such a formulation is especially suitable for parenteral administration, but may also be used for oral administration or contained in a metered dose inhaler of nebulizer for insufflation. It may be desirable to add excipients such as ethanol, polyvinylpyrrolidone, gelatin, hydroxy cellulose, acacia, polyethylene glycol, mannitol, sodium chloride or sodium citrate.

Alternatively, the compounds of the invention may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, soy bean oil, peanut oil, olive oil, glycerin, saline, ethanol, and water. Solubizing agents, such as dimethylsulfoxide, ethanol or formamide, may also be added. Carriers, such as oils, optionally with solubizing excipients, are especially suitable. Oils include any natural or synthetic non-ionic water-immiscible liquid, or low melting solid, which is capable of dissolving lipophilic compounds. Natural oils, such as triglycerides are representative. In fact, another aspect of this invention is a pharmaceutical composition comprising a compound of formula (I) and an oil.

Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. Solubilizing agents, such as dimethylsulfoxide or formamide, may also be added. The carrier may also include a sustained release material such as glyceryl monostearate or glyceryl distearate, alone or with a wax. The pharmaceutical preparations are made following the conventional techniques of pharmacy involving milling, mixing, granulating, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly p.o. or filled into a soft gelatin capsule.

For rectal administration, a pulverized powder of the compounds of this invention may be combined with excipients such as cocoa butter, glycerin, gelatin or

polyethylene glycols and molded into a suppository. The pulverized posers may also be compounded with an oily preparation, gel, cream or emulsion, buffered or unbuffered, and administered through a transdermal patch.

Nasal administration of the compounds of the invention may also be desired especially for the treatment of common cold and allergic rhinivity.

Dosing is dependent on the severity of the symptoms and on the responsiveness of the subject to the compound of the present invention. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

Example 6

Methods of Treatment with

Compounds of the Present Invention

Compounds of the present invention, when prepared according to the formulations described in Example 5, are suitable for the treatment of a number of different diseases. These diseases include those caused by the following viruses: the rhinoviruses (the common cold), enteroviruses such as poliovirus, coxsackievirus and echovirus, hepatitis A, cardioviruses such as encephalomyocarditis virus and meningovirus, and apthoviruses such as the virus for foot-and-mouth disease.

The compositions used for treatment of these diseases preferably include a pharmaceutical carrier as described in Example 5. Preferably, the compositions are administered until a defined endpoint is reached, such as the absence of a particular virus, reduction or absence of symptoms of a disease caused by the virus, or prevention of infection of the subject by the virus.

Example 7

Method of Manufacture of

a Medicament Containing an Anti-viral Compound

The following is an example of a method of manufacturing a medicament for treating a disease caused by a picornavirus. First, a compound of formula I is

SUBSTITUTE SHEET (RULE 26)

35

synthesized in accordance with good pharmaceutical manufacturing practice. Next, the compound of formula I is placed in a suitable pharmaceutical carrier, as described in Example 5 above, again in accordance with good pharmaceutical manufacturing practice.

5

Example 8

Detection of a Picornavirus

The present invention also concerns a method for the detection of picornaviral infection. According to the method of the invention a compound of the invention bearing a detectable label (for example attached to R_3), is incubated with a sample, suspected of containing picornaviruses, under conditions enabling binding of the compound to proteases. Preferably the sample should be treated with a lysing agent in order to release the picornavirus proteins from inclusion bodies. Then it is determined whether the labeled compounds of the invention are bound to any proteins in the assay. A positive answer (beyond a predetermined control level) is indicative of the presence of a picornavirus in the assayed sample.

Since the compounds of the invention may be tailored to bind specifically to a desired species of picornavirus, by modifying R_1, it is possible according to the detection method of the invention not only to determine the presence of a virus, member of the picornavirus family, in the sample but also to determine the specific species of the picornavirus to which it is bound.

It will be appreciated that the above descriptions are intended only to serve as examples, and that many other embodiments are possible within the spirit and the scope of the present invention.